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Helicobacter pylori infection in rural China: demographic, lifestyle and environmental factors

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Background Although *Helicobacter pylori* is one of the most common human bacterial infections worldwide, its mode of transmission is unclear.

Methods To investigate possible associations between *H. pylori* infection and demographic, lifestyle, and environmental factors in a rural Chinese population, a cross-sectional survey was administered to 3288 adults (1994 seropositive, 1019 seronegative, 275 indeterminate) from 13 villages in Linqu County, Shandong Province, China.

Results *Helicobacter pylori* prevalence was elevated for: infrequent handwashing before meals (OR = 1.7, 95% CI: 1.0–3.0), crowding (i.e. sharing a bed with >2 people [OR = 2.3, 95% CI: 1.3–4.2]), washing/bathing in a pond or ditch (OR = 1.5, 95% CI: 1.0–2.4), and medium (OR = 1.6, 95% CI: 1.3–2.0) and low (OR = 2.3, 95% CI: 1.9–2.9) compared to high village education level, and reduced for never being married or divorced (OR = 0.4, 95% CI: 0.2–1.0). There was also a suggestion that source of drinking water, especially water from a shallow village well might be related to *H. pylori* seropositivity. There was no evidence of an association between *H. pylori* prevalence and alcohol or tobacco use, raw fruit and vegetable intake, or individual social class measures.

Conclusions The results of this study suggest that person-to-person transmission is the most plausible route of *H. pylori* infection in this rural Chinese population, but water-borne exposures deserve further investigation.

Keywords *Helicobacter pylori*, risk factors, aetiology, transmission

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Helicobacter pylori is one of the most common human bacterial infections worldwide, but its mode of transmission is unclear. A cross-sectional survey of the 3288 adults aged 35–69 enrolled in an intervention trial in Linqu County, Shandong Province, China was conducted in 1997–1998 to assess possible risk factors that may be associated with *H. pylori* infection in this rural area of China, which has one of the highest rates of gastric cancer in the world,[1] and a greater than 60% prevalence of *H. pylori*.[1,2] Although a high percentage of the children in this area are known to be infected with *H. pylori* (50% by age 3 and 67% by age 11),[3] preliminary data indicate that seroconversions have occurred over the course of the intervention trial suggesting the possibility of new or continued infection in this cohort. A previous analysis of data from the cross-sectional study found no association between *H. pylori* prevalence and exposure to animals during childhood or adulthood and no indication of zoonotic transmission.[4] This paper explores other determinants of transmission in this high-risk area of rural China and whether an association exists between *H. pylori* seropositivity and certain demographic, environmental, and lifestyle factors.

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Methods

Study population

This study is a cross-sectional survey of 3288 *H. pylori* infected and uninfected adults from Linqu County, Shandong Province, China enrolled in a joint US National Cancer Institute (NCI)/ Beijing Institute for Cancer Research (BICR) double-blinded population-based randomized intervention trial to inhibit progression of precancerous gastric lesions. A description of the intervention trial study population and methodology have been presented in detail elsewhere.[5] In brief, all residents aged 35–69 in 1994, from 13 Linqu villages were invited to participate in a screening programme that included interview, gastroscopy, gastric biopsy, and phlebotomy. Of the 4035 possible participants, 210 subjects were excluded from the study because they were not medically eligible for endoscopy and 226 subjects were excluded because they refused endoscopy. A total of 3599 individuals completed the procedures and were eligible to participate in the intervention trial. Thirty-nine subjects refused to participate and 149 subjects were excluded because they were deceased (14), 'out-of-scope' for age or village (42), had a history of allergy to antibiotics (67), or they were not tested for antibodies to *H. pylori* (26). The remaining 3411 subjects (93% of all eligible adults in the 13 villages) were enrolled in the trial in September 1995.

Description of area

Shandong Province is one of the least economically developed provinces in China and Linqu County is one of the poorest counties in Shandong, with three-quarters of the land located in mountainous regions resulting in infertile soil and periodic severe droughts. The annual stomach cancer mortality rates for Linqu County are 70 per 100 000 for men and 26 per 100 000 for women.[1] The 13 rural villages range in size from 642 to 1845 inhabitants (mean 800). Farming is the major occupation and source of income for residents and variation in the socioeconomic status of the villages is due to differences in the ability of the land to support farming. There is also variation in the source of water, with one village using a public well, other villages using private wells, and still others using running water piped in from a village water tower. Most of the villages consist of 3–5 room brick houses built with floors of either dirt, cement, or brick. Food is stored in one room of the house. All homes have electricity, most have a small black and white TV set, and a few have telephones; however, they lack other modern conveniences such as refrigerators, washing machines, and indoor plumbing. The cooking area and pit privies/outhouses are located outside the main house.

Interview and questionnaire

To study the aetiology of *H. pylori*, in-person home interviews lasting approximately 15 minutes were conducted from October 1997 to May 1998 by trained BICR field staff. Informed consent to participate in the study was obtained from each subject prior to interview. Interviews were completed with 3288 (96%) of the 3411 study subjects enrolled in the intervention trial, and 91% of all eligible adults in the 13 villages. Reasons for non-response included death (54 subjects; 1.6%), dropped out of trial (33 subjects; 1.0%), lost to follow-up (10 subjects; 0.3%), and refusal (26 subjects; 0.8%). Neither the interviewers nor the interviewees were aware of the *H. pylori* sero-status of the study participants. The questionnaire sought information on adult socioeconomic factors, personal hygiene, and exposures during adulthood related to food, water, tobacco, alcohol, and animals. The questionnaire also collected information on certain childhood exposures related to domestic animals, household composition, type of flooring, and oral contact (eating pre-masticated food and being kissed by their parents on the lips). Data on village education were ascertained separately and used to group the 13 villages into three categories based on the average number of years of schooling residents had completed: high (range 3.99–3.83 years, 4 villages), medium (range 3.74–3.37 years, 4 villages), and low (range 3.27–2.32 years, 5 villages). Questionnaire data were merged with *H. pylori* serology test results (used as the measure of *H. pylori* infection) and information on intake of garlic and other allium vegetables obtained from the same subjects in a separate survey. The study was approved by Institutional Review Boards at BICR, Westat, Inc., and NCI.

Blood collection and *Helicobacter pylori* serological assays

In 1994, a 5-ml blood sample was collected from eligible subjects. Serum was separated and aliquoted in the field, stored immediately at –20°C, and then transferred to a –70°C freezer at BICR. One aliquot of serum (0.5 ml) was tested by one of us (LZ) for IgG and IgA antibodies to *H. pylori*.[2] *Helicobacter pylori* strains cultured from gastric biopsies of two patients in Linqu County were used to provide the antigenic preparation for serology because it had been suggested that assays based on indigenous strains had higher sensitivity and specificity.[6] Based on data from the intervention trial pilot study, the sensitivity and specificity of the two-strain Chinese assay were 90% and 87%, respectively, compared to the ^{13}C urea breath test 'gold standard'. All assays were performed on coded samples in duplicate and then repeated. The averages of the duplicate values for each of the two repeated assays were recorded. Cutoff values were based on examination of the distribution of readings in relation to a group of uninfected people and reference sera.[2] A subject was considered seropositive if both IgG optical density readings were $\geqslant$1.1 and seronegative if both IgG values were $\leqslant$0.9 and neither IgA value was $\geqslant$1.0. Otherwise, subjects were categorized as indeterminate. The *H. pylori* serostatus of the participants was positive for 1994 subjects (60.6%), negative for 1019 subjects (31.0%), and indeterminate for 275 (8.4%).

Data analysis

The measure of association used in this analysis is the prevalence odds ratio comparing the odds of being exposed to a factor given the subject was *H. pylori* seropositive to the odds of being exposed to the same factor given the subject was *H. pylori* seronegative. Maximum likelihood estimates were computed for seropositive and seronegative subjects using logistic regression.[7] Subjects with an indeterminate *H. pylori* serostatus were excluded from the analysis. Standard calculations of variances based on the logistic model that assumes that responses are independent, conditional on covariates, may be misleading in this application because 62% of the study subjects came from households with more than one participating member. Of the 2026 households represented in the study, 1110 had one participating member, 856 had two members, 49 had three members, and 11 had four members. Therefore 100 bootstrap replicates based on resampling households with replacement were used to estimate the needed variances and covariances. To control for potential confounding, age was included as a continuous variable in all logistic models. Multivariate analyses were also conducted and included age plus selected variables with significantly increased or decreased risks in the simpler models. Only the crowding-related variable 'number of people shared a bed with' was included in the multivariate model because it was highly correlated with the variable 'number of children in the household'. For each variable we present the age-adjusted prevalence odds ratio (OR), the 95% CI computed directly from the logistic regression, and/or the bootstrap 95% CI that allows for additional familial correlation. A total of 1514 males and 1499 females are included in the analyses presented. Approximately 23% of the population was aged 35–39, 26% was 40–44 years, 27% was 45–54 years, and 23% was aged 55–69.

Results

The per cent *H. pylori* seropositive decreased somewhat by age ranging from 68.8% in those aged <40 years to 64.9% in those aged 45–54 to 63.3% in those aged ⩾60. In addition, there was significant variation in *H. pylori* serostatus by village, ranging from 51.3% to 79.6% (χ^2 = 120.76, d.f. = 12, $P < 0.001$).

The association between social factors and prevalence of *H. pylori* infection is presented in Table 1. *Helicobacter pylori* seroprevalence was significantly associated with village education level. The odds of being *H. pylori* positive increased with decreasing village education level, from 1.0 for high (referent category), to 1.7 (95% CI: 1.4–2.1) for medium, to 2.4 (95% CI: 2.0–3.0) for low. There was a corresponding increase in per cent seropositive, from 54.4% to 67.2% to 74.3%. The percentage of residents with greater than a 6th grade education was 27%, 22%, and 15% for villages classified as high, medium, and low, respectively. No associations were seen for subject's level of education or annual family income. Median annual family income was 4000 yuan or approximately US$500.

Table 2 depicts the association between *H. pylori* infection and crowding/density factors and hygiene practices. The odds of being *H. pylori* positive were significantly reduced for subjects who were never married (OR = 0.4, 95% CI: 0.4–0.9), and there was a similar but non-significant reduction for the small number who were divorced. The per cent seropositive was 46.9% for never married and 40.0% for divorced compared to 66.5% for never married. Having more than one child in the household (OR = 2.0, 95% CI: 1.0–3.7) and sharing a bed with >2 people (OR = 2.1, 95% CI: 1.1–3.8) were significantly associated with the odds of being *H. pylori* seropositive. The per cent *H. pylori* seropositive was almost 80% for each of these factors. The OR was also elevated for subjects who reported washing their hands before eating less than half the time (OR = 1.6, 95% CI: 1.0–2.5; % seropositive = 74.4%) or never (OR = 3.8, 95% CI: 0.5–31.0; % seropositive = 87.5%). The odds of being *H. pylori* positive were reduced for subjects who shared cups with their family less than half the time, but no consistent patterns were seen with frequency of washing hands or body with soap, or frequency of cup washing after use (data not shown). No significant associations were noted between prevalence of *H. pylori* infection as an adult and childhood exposures (i.e. crowding or density factors, type of flooring, and oral contact) (data not shown).

Source of drinking water was found to vary between *H. pylori* positive and negative subjects (Table 3) with the highest risk (OR = 1.8, 95% CI: 1.4–2.3) and per cent seropositive (72.2%) observed for subjects who obtained their water from a shallow village well. An elevated OR was also associated with washing or bathing in a pond or ditch when the weather was warm (OR = 1.6, 95% CI: 1.0–2.4; % seropositive = 71.5%).

Table 4 presents prevalence OR for *H. pylori* infection and smoking, drinking, and dietary-related factors. The odds of being *H. pylori* seropositive among subjects who ever smoked cigarettes was slightly decreased (OR = 0.9, 95% CI: 0.7–1.0) compared to that for never-smokers, but there were no trends with lifetime number of packs smoked. There was also no association between infection status and alcohol use overall or with any measure of alcohol consumption including number of times the subject drank per week. There was a pattern of

Table 1 Association between prevalence of *Helicobacter pylori* infection and social factors

Social factors	% seropositive	No. positive	No. negative	OR[a]	95% CI	95% CI[b]
Village education level						
High	54.4	448	376	1.0	–	–
Medium	67.2	768	374	1.7	1.4–2.1	1.4–2.1
Low	74.3	778	269	2.4	2.0–2.9	2.0–3.0
Subject's education level						
<1	66.5	693	349	1.0	–	–
1–3	65.0	358	193	0.9	0.7–1.1	0.7–1.2
4–6	67.3	522	254	1.0	0.8–1.2	0.8–1.2
7–9	64.5	356	196	0.8	0.6–1.0	0.6–1.1
>9	70.7	65	27	1.1	0.7–1.8	0.7–1.8
Annual family income in Yuan[c]						
<2000	64.3	330	183	1.0	–	–
2000–3999	67.8	555	264	1.1	0.8–1.3	0.8–1.3
4000–5999	65.2	421	225	0.9	0.7–1.2	0.7–1.2
6000–7999	63.3	269	156	0.8	0.6–1.1	0.6–1.1
>8000	68.6	412	189	1.0	0.8–1.4	0.8–1.4

[a] Odds ratio adjusted for age in a logistic model.

[b] Calculated using bootstrap technique.

[c] 4000 yuan = approximately US$500.

Table 2 Association between prevalence of *Helicobacter pylori* infection and crowding/density factors and hygiene practices

Factor	% seropositive	No. positive	No. negative	OR[a]	95% CI	95% CI[b]
Marital status						
Married	66.5	1831	921	1.0	–	–
Widowed	65.2	146	78	1.1	0.8–1.4	0.8–1.5
Divorced	40.0	2	3	0.4	0.06–2.2	NA[c]
Never married	46.9	15	17	0.4	0.2–0.9	0.2–0.9
No. of children in household						
0	65.5	1458	767	1.0	–	–
1	67.2	402	196	1.0	0.9–1.3	0.8–1.3
>1	79.7	55	14	2.0	1.1–3.6	1.0–3.7
No. of people shared a bed with						
0	63.0	283	166	1.0	–	–
1	67.2	1309	640	1.1	0.9–1.4	0.9–1.4
2	62.9	254	150	0.9	0.7–1.2	0.6–1.2
>2	79.8	67	17	2.1	1.2–3.7	1.1–3.8
Frequency of handwashing before eating meals						
Always	66.1	1689	867	1.0	–	–
> half the time	64.6	237	130	1.0	0.8–1.2	0.7–1.2
< half the time	74.4	61	21	1.6	1.0–2.6	1.0–2.5
Never	87.5	7	1	3.8	0.5–31.0	NA[c]
Frequency of sharing cups with family						
Always	66.9	1847	915	1.0	–	–
> half the time	62.0	31	19	0.9	0.5–1.5	0.4–1.8
< half the time	51.1	24	23	0.6	0.3–1.0	0.3–1.1
Never	59.7	92	62	0.8	0.6–1.1	0.6–1.1

[a] Odds ratio adjusted for age in a logistic model.

[b] Calculated using bootstrap technique.

[c] Not available because the sample size was too small.

Table 3 Association between prevalence of *Helicobacter pylori* infection and water-related factors

Environmental factor	% seropositive	No. positive	No. negative	OR[a]	95% CI	95% CI[b]
Source of drinking water						
Deep private well	59.0	235	163	1.0	–	–
Shallow private well	64.9	1004	543	1.3	1.0–1.6	1.0–1.7
Deep village well	66.7	70	35	1.4	0.9–2.2	0.8–2.3
Shallow village well	72.2	648	250	1.8	1.4–2.3	1.4–2.3
River	53.2	25	22	0.8	0.4–1.5	0.4–1.5
Running/spring water	66.7	12	6	1.4	0.5–3.8	0.6–3.4
Frequency that water is boiled						
Always	66.1	1372	704	1.0	–	–
Often	66.7	511	255	1.0	0.8–1.2	0.8–1.2
Sometimes	70.0	77	33	1.1	0.8–1.7	0.8–1.7
Never	55.9	33	26	0.7	0.4–1.1	0.4–1.1
Location washed/bathed when the weather was warm						
Never washed	59.9	218	146	1.0	–	–
Washed in pond/ditch	71.5	108	43	1.6	1.0–2.4	1.0–2.4
Washed elsewhere	66.8	1668	829	1.3	1.0–1.6	1.0–1.6

[a] Odds ratio adjusted for age in a logistic model.

[b] Calculated using bootstrap technique.

decreasing OR with increased consumption of allium vegetables (ranging from 1.0 for <5.5 kg per year to 0.8 for >10.5 kg per year), but the trend was not significant. There was no apparent association between *H. pylori* infection and the number of times per month raw fruits and vegetables were consumed. Overall, there was no association between ever having gastric reflux and prevalence of *H. pylori* infection. However, the OR and the per cent seropositive were slightly but non-significantly reduced

Table 4 Association between prevalence of *Helicobacter pylori* infection and smoking, drinking, and dietary factors

Exposure	% seropositive	No. positive	No. negative	OR[a]	95% CI	95% CI[b]
Lifetime packs of cigarettes smoked						
Never smoked	67.8	1166	553	1.0	–	–
Ever smoked	64.0	828	466	0.9	0.7–1.0	0.7–1.0
<3595	63.3	164	95	0.8	0.6–1.1	0.7–1.0
3595–6862	69.0	176	79	1.1	0.8–1.4	0.8–1.4
6863–9490	64.9	181	98	0.9	0.7–1.1	0.7–1.1
9491–14 235	60.3	152	100	0.7	0.6–1.0	0.6–1.0
>14 235	62.1	154	94	0.8	0.6–1.1	0.6–1.1
No. of times per week drank alcohol						
Never drank	66.6	1092	548	1.0	–	–
Ever drank	65.7	902	471	1.0	0.8–1.1	0.8–1.1
<3	68.0	217	102	1.0	0.8–1.3	0.8–1.4
3–6	66.2	229	119	1.0	0.7–1.2	0.7–1.2
7–9	65.0	271	146	0.9	0.8–1.2	0.7–1.2
>9	63.8	183	104	0.9	0.7–1.2	0.7–1.2
Kg of allium vegetables eaten per year						
0–5.0	68.7	354	161	1.0	–	–
5.5–7.5	66.5	342	172	0.9	0.7–1.1	0.7–1.1
8–10.5	66.2	339	173	0.9	0.7–1.1	0.6–1.2
11–15	64.9	361	195	0.8	0.6–1.1	0.6–1.0
>15	65.6	319	167	0.8	0.6–1.1	0.7–1.1
No. of times per month ate raw fruits and vegetables						
Never ate	65.6	103	54	1.0	–	–
Ever ate	66.2	1891	964	1.0	0.7–1.4	0.7–1.4
<3	66.4	296	150	1.0	0.7–1.5	0.7–1.5
3–5.9	63.8	391	222	0.9	0.6–1.3	0.6–1.3
6–9.9	65.1	408	219	0.9	0.6–1.3	0.6–1.4
10–15.9	68.5	438	201	1.1	0.7–1.6	0.7–1.6
⩾16	67.5	358	172	1.0	0.7–1.5	0.7–1.5
No. of times per month have gastric reflux						
Never had	66.2	1089	555	1.0	–	–
Ever had	66.0	901	464	1.0	0.8–1.1	0.8–1.1
<0.43	65.8	300	156	1.0	0.8–1.2	0.8–1.2
0.43–3.9	68.4	357	165	1.1	0.9–1.3	0.9–1.3
4.0–8.9	65.0	117	63	0.9	0.7–1.3	0.7–1.4
⩾9	61.4	127	80	0.8	0.6–1.1	0.6–1.1

[a] Odds ratio adjusted for age in a logistic model.

[b] Calculated using bootstrap technique.

for subjects who had reflux nine or more times per month (OR = 0.8, 95% CI: 0.6–1.1; % seropositive = 61.4%).

Table 5 presents OR for variables of interest adjusted for one another in multivariate logistic models using bootstrap techniques to calculate CI. The OR for subjects who obtained their water from a shallow village well was significantly elevated in Model I (OR = 1.8, 95% CI: 1.4–2.3), but was no longer significantly elevated in Model II (OR = 1.2, 95% CI: 0.9–1.6) when village education level was included. The adjusted OR for village education level was 1.6 (95% CI: 1.3–2.0) for subjects living in a village classified as medium and 2.3 (95% CI: 1.9–2.9) for those living in a village classified as low. Odds ratios in Model II were also elevated for subjects who shared a bed with >2 people (OR = 2.3, 95% CI: 1.3–4.2), for those who washed their hands before meals less than half the time (OR = 1.7, 95% CI: 1.0–3.1), and for those who washed in a pond or ditch (OR = 1.5, 95% CI: 1.0–2.4). The OR was reduced for those who never married or were divorced (OR = 0.4, 95% CI: 0.2–1.0).

Discussion

This large cross-sectional survey in Shandong Province, China provided us the opportunity to study everyone in the population aged 35–69 to determine what factors were related to *H. pylori* positivity. Social class factors have been associated with *H. pylori* infection status in a number of studies throughout the world.[8–18] In this study, we found a strong inverse relationship between village education level and prevalence of *H. pylori* infection, but no association with subject's education level and

Table 5 Association between prevalence of *Helicobacter pylori* infection and selected factors in a multivariate logistic model

Selected factors	Model I[a]		Model II[b]	
	OR[c]	95% CI	OR	95% CI
Marital status (compared to married)				
Widowed	1.0	0.7–1.5	1.0	0.7–1.6
Never married or divorced	0.4	0.2–0.9	0.4	0.2–1.0
No. of people shared a bed with (compared to none)				
1	1.1	0.9–1.4	1.1	0.9–1.4
2	0.8	0.6–1.2	0.9	0.7–1.3
>2	2.0	1.1–3.6	2.3	1.3–4.2
Frequency of handwashing before eating meals (compared to always)				
> half the time	1.0	0.7–1.2	0.9	0.7–1.2
< half the time	1.8	1.0–3.1	1.7	1.0–3.0
Location washed/bathed when the weather was warm (compared to never washed)				
Washed in pond or ditch	1.3	1.0–1.6	1.2	0.9–1.6
Washed elsewhere	1.6	1.0–2.4	1.5	1.0–2.4
Source of drinking water (compared to deep private well)				
Shallow private well	1.2	1.0–1.6	1.0	0.8–1.3
Deep village well	1.3	0.8–2.1	1.0	0.6–1.6
Shallow village well	1.8	1.4–2.3	1.2	0.9–1.6
River or running/spring water	0.9	0.5–1.5	0.5	0.3–0.9
Village education level (compared to high)				
Medium	–	–	1.6	1.3–2.0
Low	–	–	2.3	1.9–2.9

[a] Multivariate logistic model used bootstrap technique and contained the following variables: age, marital status, number of people shared a bed with, hand washing before meals, location washed/bathed, and source of drinking water.

[b] Multivariate logistic model used bootstrap technique and contained the above variables plus village education level.

[c] Odds ratio.

annual family income. It is possible that average village education level in rural China may reflect general lifestyle or hygiene practices that vary substantially between villages. For example, there was some suggestion in our data that source of drinking water may be related to *H. pylori* infection, with the greatest risk observed for subjects who obtained their water from a shallow village well. However, since water source is highly correlated with village education level (e.g. the poorest and least educated villages obtain their water from a shallow village well) it is possible that some of the excess risk seen for medium and low village education level may be due to differences in water source. Evidence that waterborne transmission may be important, especially in areas of the world with high rates of *H. pylori* infection and less than adequate water quality comes from studies conducted in Colombia, China, Peru, Japan, and Inuit communities in Canada.[2,19–23]

We found elevated OR associated with measures of crowded living conditions, particularly number of children in the household and the number of people sharing a bed, and a reduced risk for subjects who never married or were divorced. These findings are consistent with other recent studies[10,11,19,24–30] and suggest that household transmission contributes to *H. pylori* seropositivity.

We sought information about the frequency as an adult of handwashing and found elevated OR associated with infrequent handwashing before meals, but no protective effect of washing hands or body with soap or detergent. A recent study in rural Guatemala found evidence of *H. pylori* DNA under the fingernails of infected subjects, suggesting that the hand may play a role in transmission.[31] Several studies found the prevalence of *H. pylori* infection to be higher in subjects who did not have a bathroom, indoor toilet, or running water.[26,32,33] These modern necessities were not found in any of the homes of our study subjects. Since all of our study subjects ate with chopsticks, we also could not confirm the excess risk (OR = 2.5) reported among Chinese immigrants in Australia who used chopsticks.[12]

Analysis of the relationship between smoking and *H. pylori* infection in recent studies has provided conflicting results. While some studies have reported significantly elevated risks of *H. pylori* infection with smoking[8,30,34–38] and one reported significantly reduced risks,[39] most, similar to ours, found no significant association with current smoking or any other measure of cigarette use.[2,12,14,14,17,24,40–45] Recent epidemiological studies that looked at the relation between alcohol use and *H. pylori* infection reported either no association[11,12,14,14,18,36,41] or a reduction in risk[8,17,37,39,46–48] that was stronger for wine than beer and more apparent at moderate to high levels of alcoholic beverage consumption. Similarly, our study found no association between *H. pylori* infection and ever/never use of alcohol, and only a slight (OR = 0.9) non-significant reduction in the OR for subjects who drank alcohol ⩾7 times per week. The relative homogeneity among drinkers in our study, including the lack of heavy drinkers, may have limited our ability to detect a protective effect from alcohol's antimicrobial properties.[49] The use of serostatus as a marker of infection could also mask a potential association of alcohol consumption and

H. pylori infection as antibodies are still circulating in the blood after accidental *H. pylori* loss. Increased consumption of allium vegetables has been suggested as a protective factor for stomach cancer[50,51] and its precursor lesions,[52] and garlic has been shown to inhibit *H. pylori in vitro,*[53,54] but not *in vivo*.[55] In our study, we found a pattern of decreasing OR with increased consumption of all allium vegetables combined, although the trend was not significant. Several studies have reported significantly reduced OR and negative gradients in risk of *H. pylori* infection with increased consumption of fruits and/or vegetables, vitamin C, and beta-carotene.[19,37,56,57] In contrast, consumption of raw/uncooked vegetables was associated with elevated risk of *H. pylori* infection.[19,58] In our data, there was no apparent association between *H. pylori* infection and the number of times per month raw fruits and vegetables were eaten.

Some studies have suggested that *H. pylori* infection appears to be lower in people with gastroesophageal reflux disease (the major risk factor for Barrett's oesophagus which is strongly associated with adenocarcinoma of the oesophagus) than in controls.[59,60] In our study, we found slight non-significant protective effects for subjects who reported the occurrence of gastric reflux nine times or more per month.

The major strengths of this cross-sectional investigation are that the data were sought from all eligible individuals age 35–69 in 13 rural Chinese villages, participation rates were high, and *H. pylori* status (unknown by study investigators, interviewers, and subjects) was determined objectively based on the ELISA optical density test utilizing an indeterminate zone (± 10% of the cutoff value of 1.0). In addition, the same structured questionnaire was administered to all study subjects by trained interviewers and bootstrap CI were calculated to avoid underestimation of the standard errors due to possible intrafamilial correlations. Although there may be some degree of misclassification, inaccuracies in data acquisition are not likely to differ systematically according to *H. pylori* serostatus because of the blinded design.

There are also several limitations of this study. The outcome was measured as prevalence of disease and thus reflects *H. pylori* infections that could have occurred from early childhood to several weeks before the blood sample was drawn; the suboptimal sensitivity and specificity (90% and 87%, respectively) of the serological test would tend to attenuate the risk estimates making it more difficult to find associations; the outcome and the exposure were assessed simultaneously, making it impossible to determine temporal relationships; and the high degree of homogeneity in this rural Chinese population may have limited the ability to detect differences between *H. pylori* positive and negative subjects.

In summary, our data from this rural Chinese population suggest a possible association between *H. pylori* and village education level, marital status, crowding, lack of handwashing, washing/bathing in a pond or ditch, and source of drinking water, but no evidence of an association between *H. pylori* and alcohol or tobacco use, raw fruit and vegetable intake, or individual social class measures. Based on these findings, person-to-person transmission appears to be the most plausible route for *H. pylori* infection in this rural Chinese population, but waterborne exposures deserve further investigation.

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Commentary: What remains to be done regarding transmission of *Helicobacter pylori*

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Helicobacter pylori causes a chronic and serious infection. *Helicobacter pylori* shares with syphilis and tuberculosis the features of a long latent period, a small proportion of infected individuals experiencing clinical illness, and a male predominance of clinical disease despite equal infection among sexes. Humans are the only known reservoir. Transmission is 'opportunistic' in that any method that allows the organism access to the stomach is likely to be a mode of transmission. The most common modes of transmission involve lapses in household hygiene, or ingestion of contaminated food or water. The infection is typically acquired in childhood or when children are in the family.[1] In developed countries the prevalence of the infection is falling in all adult birth cohorts (loss exceeds the rate of acquisition of the infection).[2,3] This is possibly related to the widespread use of antibiotics for other infections with the general high levels of hygiene practised limiting reacquisition. Prior to the culture of *H. pylori*, gastritis was an important topic for epidemiological studies and many features and associations were subsequently confirmed. What new epidemiological information is needed and do the three papers in this issue address these questions?[4–6]

Moayyedi *et al.* examined several risk factors for *H. pylori* acquisition among adults between the ages of 40–49 years from an urban community in the north of England.[4] The original design was an intervention trial to evaluate the outcome of medical benefits on *H. pylori* eradication and it also provided data for epidemiological analysis. Although the participants were randomly selected, the sample was not representative of the general population in terms of social class and frequency of dyspepsia. *Helicobacter pylori* is typically acquired in childhood making it unlikely that typical adult lifestyles would influence the risk of *H. pylori* infection. The authors confirmed prior observations that smoking, alcohol and coffee consumption, and usual adult lifestyles are not risk factors for *H. pylori* acquisition, whereas childhood socioeconomic status and overcrowding are.

Brown *et al.* report a cross-sectional study from a rural population between the ages of 35 and 69 from Shandong Province, China.[5] This study was a portion of a larger trial of cancer prevention and was not designed to specifically address *H. pylori* transmission. Prior studies from China had not found a role for water in *H. pylori* transmission, possibly because of the widespread practice of boiling water before consumption.[7] This study suggested that drinking water from shallow wells in the villages might lead to *H. pylori* acquisition. Unfortunately, because the water source was highly correlated with the educational level of the village, it was not possible to separate an excess risk for *H. pylori* acquisition related to differences in water source. Overall, there are sufficient studies that strongly support a role for water in *H. pylori* transmission, but only in some localities. A recent study from Kazakhstan found a significant association between the prevalence of *H. pylori* infection and household hygienic variables related to water use.[8] Further studies in areas with a high prevalence of *H. pylori* infection, where water is likely a source of infection, are needed to investigate the variables to show how the water source plays a role in transmission. Such studies must include important household sanitary variables related to water source, water storage and use.

A large population-based study of the relationship between breastfeeding and *H. pylori* infection was conducted in Germany. The study was one of a series of 'minimal publishable units' from this group drawn from larger studies. This study targeted First Grade children. The authors reported no protective effect of breastfeeding and found a trend for a higher rate of infection among those who were breastfed. *Helicobacter pylori* infection was concentrated among families in which the mother was also infected (exposure). These results contrasted sharply with another large population study of non-affluent Black and Hispanic children in the US that reported a consistently lower rate of *H. pylori* infection among breastfed children compared to those not breastfed, independent of the mothers' educational level.[9] There were several potential technical flaws in the German study that might influence the accuracy of detection of *H. pylori* infection including failure to normalize the results of the breath test for CO_2[10] and use of an infrared spectrometer which is inherently less accurate than mass spectroscopy near

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the cut-off value. Additionally, the relatively long delay between the breastfeeding period and assessment of *H. pylori* status allowed any behavioural differences linked with the tendency to breastfeed to become confounding variables. Subsequent studies are needed that focus on behavioural differences in relation to breastfeeding practices (moistening the nipple with saliva) and social/ethnic differences such as premastication to understand why the results among children living in Germany were different than among children in the US.

Helicobacter pylori infection clusters within families and improvement in socioeconomic status of parents reduces the risk and rate of transmission. Transmission requires exposure to an infected person or contaminated source as well as access of the organism to the stomach. The evidence overwhelmingly supports person-to-person transmission as the predominant mechanism. In areas with lax sanitation, contaminated water or food may also play a role in transmission. To date, most studies have examined surrogates such as socioeconomic status instead of attempting to break down and identify the elements of household hygiene that are critical elements within a particular society or ethnic group. Enteric infection related to water can be either water-borne (such as cholera), or related to poor sanitary practices (water-washed), or both. The data suggest that *H. pylori* can be transmitted either way, and that to reduce the rate of transmission, an improvement in overall sanitation will be required, including clean water, waste disposal, and household hygienic practices. Unfortunately, the extraction of an epidemiological 'study' from a study with a different primary focus rarely provides little in the way of new information or insights. None of these three studies discussed above address the critical issues. The remaining critical issue is to identify the weak links in the chain of transmission amenable to behavioural modification.

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